

DATA EVALUATION RECORD
WHOLE SEDIMENT ACUTE TOXICITY INVERTEBRATES, MARINE
OPPTS Guideline 850.1740

1. **CHEMICAL:** Pyraclostrobin

PC Code No.: 099100

2. **TEST MATERIAL:** [^{14}C]BAS 500 F
BAS 500 F

Radiochemical Purity: 99.4%
Purity: 99.02%

3. **CITATION:**

Authors: Faertner, K.

Title: BAS 500 F: Whole Sediment Acute Toxicity to a Marine Amphipod (*Leptocheirus plumulosus*).

Study Completion Date: January 15, 2013

Laboratory: ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, MO 65202

Sponsor: BASF Corporation
26 Davis Drive
Research Triangle Park, NC 27709

Laboratory Report ID: 68249

MRID No.: 49080402

DP Barcode: 410997

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, CSS-Dynamac Corporation

Signature: 

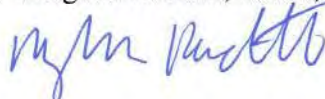
Date: 06/28/13

APPROVED BY: John Marton, Ph.D., Environmental Scientist, CDM Smith

Signature: 

Date: 11/09/13

5. **APPROVED BY:** Meghan Radtke, Ph.D., Biologist

Signature: 

Date: 12/9/13

6. **STUDY PARAMETERS:**

Age of Test Organism:	2-4 mm
Definitive Test Duration:	10 days
Study Method:	Static, with aeration
Type of Concentrations:	Mean-measured sediment (bulk and OC-normalized), pore and overlying water

7. CONCLUSIONS:**Results Synopsis:**Based on mean-measured sediment concentrations:

LC ₅₀ : 4.35 mg TRR/kg	95% C.I.: 3.53-5.23 mg TRR/kg
Slope: 8.72	95% C.I.: 3.46-14
NOAEC: 2.74 mg TRR/kg	
LOAEC: 5.05 mg TRR/kg	

Based on mean-measured OC-normalized sediment concentrations (TOC= 0.00147%):

LC ₅₀ : 295,918 mg TRR/kg	95% C.I.: 240,136-355,782 mg TRR/kg
Slope: 8.72	95% C.I.: 3.46-14
NOAEC: 186,395 mg TRR/kg	
LOAEC: 343,537 mg TRR/kg	

Based on mean-measured pore water concentrations:

LC ₅₀ : 0.0099 mg TRR/L	95% C.I.: 0.00878-0.011 mg TRR/L
Slope: 9.02	95% C.I.: 5.42-12.6
NOAEC: 0.00625 mg TRR/L	
LOAEC: 0.0115 mg TRR/L	

Based on mean-measured overlying-water concentrations:

LC ₅₀ : 0.00461 mg TRR/L	95% C.I.: 0.00402-0.0052 mg TRR/L
Slope: 8.4	95% C.I.: 4.88-11.9
NOAEC: 0.00282 mg TRR/L	
LOAEC: 0.00541 mg TRR/L	

8. ADEQUACY OF THE STUDY:

A. Classification: Acceptable

B. Rationale: N/A

C. Repairability: N/A

9. MAJOR GUIDELINE DEVIATIONS:

There were no major guideline deviations.

10. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: <i>Ampelisca abdita</i> , <i>Eohaustorius estuarius</i> , <i>Leptocheirus plumulosus</i> , or <i>Rhepoxynius</i> <i>abronius</i>	<i>Leptocheirus plumulosus</i>
Life Stage: 2-4 mm for <i>L. plumulosus</i> to 3-5 mm for the three remaining species. No mature male or female <i>A. abdita</i> or <i>L. plumulosus</i> should be used for testing.	2-4 mm
Supplier The methods for collection of test organisms are species-specific. Organisms obtained from the wild are permissible.	Chesapeake Cultures Hayes, VA
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period:	Upon receipt, the organisms were held for 3 days prior to testing.
Feeding:	During the holding period, amphipods were fed a flake food suspension.
Pretest Mortality:	None

C. Test System

Guideline Criteria	Reported Information
<p>Source of dilution water (overlying water) and sediment: Natural sea water is preferable, but reconstituted water is acceptable. Natural sea water should be from an uncontaminated source, covered, maintained at 4°C, and used within 2 days of collection.</p> <p>Uncontaminated natural sediment is recommended, but formulated sediment is acceptable. Natural sediments should be stored at 4°C in the dark and used within 2 to 8 weeks of collection.</p>	<p>The dilution water was laboratory saltwater prepared using a commercial sea salt mix (Crystal Sea Marinemix, Marine Enterprises International, Inc., Baltimore, MD) and laboratory freshwater to achieve a final salinity of $20 \pm 2\%$. The laboratory freshwater consisted of well water that was de-mineralized by reverse-osmosis to yield water with a total hardness of 130 to 160 mg CaCO₃/L. The prepared dilution water was passed through a 5 µm filter and UV-sterilized prior to use.</p> <p>Natural marine sediment (Lot No. MSed101012) was collected from Sequim Bay, WA on October 8, 2012, stored at <i>ca.</i> 4°C, and sieved (0.5-mm) prior to use.</p>
<p>Does water support test animals without observable signs of stress?</p>	<p>Yes</p>
<p>Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L</p>	<p>There were no apparent problems with water quality.</p>
<p>Water Temperature 15°C for <i>E. estuarius</i> and <i>R. abronius</i>, 20°C for <i>A. abdita</i>, and 25°C for <i>L. plumulosus</i>.</p>	<p>24.3 to 25.3°C</p>
<p>pH</p>	<p>7.6 to 8.5</p>
<p>Dissolved Oxygen Results are unacceptable if DO falls to <60% saturation.</p>	<p>4.3 to 7.6 mg/L (60 to 105% saturation)</p>

Guideline Criteria	Reported Information
Salinity Overlying water salinity should be 28 ppt for <i>A. abdita</i> and <i>R. abronius</i> and 20 ppt for <i>E. estuarius</i> and <i>L. plumulosus</i> .	19.0 to 21.0‰
Ammonia	0.00506 to 0.259 mg/L
Sediment Characterization All sediment must be characterized for: pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content.	Particle distribution – 27% sand, 55% silt, and 18% clay USDA classification – silt loam Organic matter content – 6.4% Organic carbon content – 14.7 ppm (0.00147%) Moisture – 199.6% (as received; dw basis) Pore water salinity – 27.0 to 29.5‰ Pore water pH – 7.3 to 7.5
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, total inorganic carbon, total volatile solids, acid volatile sulfides, metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	<u>Day-0 Pore water analysis:</u> Temperature: 24.2 to 24.5°C pH: 7.7 to 7.8 Un-ionized ammonia: 0.233 to 0.425 mg/L

Guideline Criteria	Reported Information
<p>Effect of Grain Size Grain size may adversely affect some species of amphipod:</p> <p><i>A. abdita</i>: survival may be impacted in sediments containing 95% or more sand.</p> <p><i>L. plumulosus</i>: survival should not be impacted in clean sediments containing 100% sand to 100% sand + clay.</p> <p><i>E. estuarius</i>: survival is unaffected by clean sediments containing 0.6 to 100% sand. However, increased mortality may be associated with increased proportions of fine-grained sediment.</p> <p><i>R. abronius</i>: very fine grains, particularly silts and clays, may reduce survival.</p>	<p>As 99 and 98% survival was observed in the negative and solvent control group, respectively, sediment grain size did not adversely affect survival of this species.</p>
<p>Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.</p>	<p><u>Test substances:</u></p> <p>1) BAS 500 F Synonym: pyraclostrobin CAS no.: Batch no.: COD-001236 Description: tan, viscous solidified melt Purity: 99.02% Storage: room temperature</p> <p>2) [¹⁴C]BAS 500 F Lot no.: 579-6009 Radiochemical purity: 99.4% Specific activity: 64.4 MBq/g Storage: ca. -20°C</p>

Guideline Criteria	Reported Information
<p>Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.</p>	<p>A non-labeled primary stock solution was prepared in acetone at 5.06 mg ai/mL. The primary dosing solution was prepared by combining 12.1 mL of radiolabeled test material with 9.78 mL of the unlabeled stock solution and bringing to 25 mL with acetone: the final concentration of the dosing solution was 2.00 mg ai/L. The primary dosing solution and dilutions in acetone were used to dose the sediment.</p> <p>A solvent control and negative control group were included in the test.</p>
<p>Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.</p>	<p>A 10-mL volume of the appropriate dosing solution was mixed into 100 g of air-dried fine silica sand in separate glass jars. The jars were placed in a fume hood overnight to allow the acetone to completely evaporate. Following removal of the solvent, the carrier sand was hand-mixed into 5602.2 g of sediment (dw equivalent of 1900 g).</p> <p>The range of concentrations (0.63 to 10 mg ai/kg dw sediment) were selected based upon the results of a preliminary range-finding study.</p>
<p>Test Aquaria 1. <u>Material</u>: Glass or stainless steel or perfluorocarbon plastics. 2. <u>Size</u>: 1 L chambers containing 175 ml (2 cm) of sediment and 800 ml of overlying water.</p>	<p>1. Glass jar</p> <p>2. 1 L (17 cm high x 8.5 cm diameter), containing ca. 175 mL (ca. 251.8 g) of sediment and 600 mL of overlying water.</p>
<p>Type of Dilution System Static</p>	<p>Static</p>

Guideline Criteria	Reported Information
Aeration Overlying sea water should be continuously aerated except when test organisms are being added. DO should be maintained at approximately 90% saturation using gentle aeration without disturbing the sediment.	Aeration was provided at an initial rate of 60 to 100 bubbles per minute to each chamber through a glass pipet. Aeration was discontinued during the insertion of the animals, and resumed within 50 minutes after addition was completed. The aeration was adjusted as necessary to maintain the DO concentrations at $\geq 60\%$ saturation.
Photoperiod Constant lighting to assure that the test organisms remain burrowed. Recommended intensity is 500 to 1000 lux.	Continuous lighting; 501 to 601 lux
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used, it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	Acetone, 10 mL/2000 total dw sediment The solvent was completely evaporated from the sand carrier prior to incorporation into the sediment.

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.	One day prior to the addition of the amphipods (day -1), the test systems were established. Overlying water was gently added using a deflector to minimize disturbance of the sediment, and the vessels were placed under test conditions.

Guideline Criteria	Reported Information
Renewal of Overlying Water: The overlying water does not have to be renewed.	None
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	Amphipods were impartially assigned to intermediate test containers, and each container was randomly assigned to one treatment replicate. The test was initiated when amphipods from each container were released into their corresponding treatment replicate vessel.
Range Finding Test A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment.	<u>Preliminary toxicity assessment</u> <ul style="list-style-type: none"> • Amphipods received from Chesapeake Cultures 2 days prior to test initiation • Natural marine sediment collected on April 16, 2012 • 10-day exposure initiated April 28, 2012 at nominal levels of 0 (negative and solvent controls), 0.010, 0.10, 1.0, 10, 100 and 1000 mg ai/kg dw sediment • 20 amphipods per level • Survival averaged 0 (negative control), 0 (solvent control), 0, 5, 0, 100, 100 and 100%, respectively
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.	Observations of mortality and general health/behavior were assessed at study termination (Day 10).
Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.	0 (negative control), 0 (solvent control), 0.63, 1.3, 2.5, 5.0 and 10 mg ai/kg dw sediment Aqueous solubility was not reported.

Guideline Criteria	Reported Information
<p>Number of Test Organisms 20 organisms per test chamber are recommended. Five replicates per treatment should be used.</p>	<p>160 amphipods per level, with 20 amphipods per replicate beaker, and eight biological replicates per level</p> <p>An additional four replicates were maintained for chemical analysis, and an additional two replicates were used for initial and terminal pore water ammonia analysis.</p>
<p>Test organisms randomly or impartially assigned to test vessels?</p>	<p>Yes</p>
<p>Feeding Organisms do not have to be fed during the test.</p>	<p>None reported</p>
<p>Water Parameter Measurements Temperature should be measured daily from at least one replicate per treatment, and must be monitored continuously in water bath or exposure chamber.</p> <p>Salinity, DO, and pH should be measured in overlying water daily in one test chamber per treatment, and should be measured in all test chambers at study initiation and termination.</p> <p>Ammonia should be measured near day 2 and day 8, and should be accompanied by pH and temperature measurements.</p> <p>pH, temperature, and ammonia concentration should be measured in pore water at the beginning of the test.</p>	<p><u>Overlying water:</u> DO, temperature, pH, and salinity were measured in each replicate vessel on Days 0 and 10, and in one replicate vessel daily. The temperature of the water bath was also continuously monitored. Ammonia was measured at each level from composite samples on Days 0 and 10.</p> <p><u>Pore water:</u> Ammonia samples were measured from the additional replicates prepared for each treatment at Days 0 and 10.</p>

Guideline Criteria	Reported Information
Chemical Analysis Concentrations should be measured in sediment, interstitial water, and overlying water at the beginning and end of the test. Degradation products should also be measured where appropriate.	Sediment and pore water were isolated using centrifugation (4000 rpm for 20 minutes at 20°C). <u>Total Radioactive Residues (TRR)</u> On Days 0 and 10, overlying water and pore water from all levels were analyzed for TRR of pyraclostrobin using LSC, and associated sediment was analyzed for TRR of pyraclostrobin using LSC following combustion. <u>Characterization of TRR</u> Overlying water from the 0.63 mg ai/kg level was further analyzed for pyraclostrobin using HPLC (see Reviewer's Comments section). The radioactivity in sediment and pore water was not further characterized.

11. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in accordance with GLP Standards as specified in 40 CFR 160 with the following exception: the latest water characterizations performed in February 2011.
Control Criteria Was recovery of organisms from control sediment should equal or exceed 90% in a 10-day test or 80% in a 28-day test?	Yes, survival averaged 99 and 98% for the negative and solvent control groups, respectively

Guideline Criteria	Reported Information
Percent Recovery of Chemical:	<p>The following results were based upon QC samples fortified and analyzed concurrently with the test samples.</p> <p><u>Sediment</u> LSC – 96 to 106% (LOQ = 0.0160 to 0.0192 mg TRR/kg dw sediment)</p> <p><u>Overlying Water</u> LSC – not reported (LOQ = 0.000664 to 0.000684 mg TRR/L) HPLC – 75 to 110% (LOQ = 0.0000800 mg ai/L)</p> <p><u>Interstitial Water</u> LSC – not reported (LOQ = 0.0000664 to 0.0000684 mg TRR/L)</p>
Data Endpoints - Survival - Reburial, optional for <i>E. estuarius</i> , <i>L. plumulosus</i> , and <i>R. abronius</i> .	- Survival
Raw data included?	Yes, sufficient

Effects Data

Toxicant Concentration				Percent Survival
Nominal (mg/kg)	Mean-Measured			
	Sediment (mg TRR/kg)	Pore Water (mg TRR/L)	Overlying Water (mg TRR/L)	
Negative Control	<LOQ ^(a)	<LOQ ^(b)	<LOQ ^(b)	99
Solvent Control	<LOQ ^(a)	<LOQ ^(b)	<LOQ ^(b)	98
0.63	0.771	0.00169	0.000526	98
1.3	1.51	0.00338	0.00136	100
2.5	2.74	0.00625	0.00282	97
5.0	5.05	0.0115	0.00541	25*
10	11.3	0.0229	0.0115	0.63*

^(a) LOQ = 0.0192 mg TRR/kg dw sediment on Day 0, and 0.0160 mg TRR/kg dw sediment on Day 10.

^(b) LOQ = 0.000684 mg TRR/L on Day 0, and 0.000664 mg TRR/L on Day 10.

* Statistically significant reduction compared to the negative control value (Fisher's One-tailed exact test).

Other Significant Results:

Biological: Survival after 10 days averaged 99 and 98% for the negative and solvent control levels, respectively, compared to 98, 100, 97, 25 and 0.63% for the mean-measured 0.771, 1.51, 2.74, 5.05 and 11.3 mg TRR/kg sediment levels, respectively. Differences were statistically-significant ($p < 0.05$) compared to the negative control at the mean-measured 5.05 and 11.3 mg TRR/kg levels.

Based on mean-measured TRR sediment levels, the 10-day LC_{50} (with 95% C.I.) was 4.412 (4.192 to 4.643) mg TRR/kg, and the NOAEC and LOAEC were 2.74 and 5.05 mg TRR/kg, respectively.

Analytical: Overlying water, pore water, and sediment samples were analyzed on Days 0 and 10 for total radioactive residues (TRR) of BAS 500 F (pyraclostrobin). In addition, overlying water samples from the 0.63 mg ai/kg level were also analyzed for pyraclostrobin on Days 0 and 10.

Results indicated that the majority of TRR remained associated with the sediment phase; mean-measured concentrations ranged from 101 to 122% of nominal concentrations for all levels. In overlying water, TRR increased very slightly at all levels from Days 0 to 10, with mean-measured concentrations of 0.000526, 0.00136, 0.00282, 0.00541 and 0.0115 mg TRR/L for the nominal 0.63, 1.3, 2.5, 5.0 and 10 mg ai/kg dw sediment levels, respectively. Measured concentrations in two replicates of the 0.63 mg ai/kg level at Day 0 were 0.000128 and 0.000705 mg ai/L. At termination, the concentrations were 0.000342 and 0.0000492 mg ai/L. Concentrations in the pore water decreased at all levels during the study, with mean-measured concentrations of 0.00169, 0.00338, 0.00625, 0.0115 and 0.0229 mg TRR/L for the nominal 0.63, 1.3, 2.5, 5.0 and 10 mg ai/kg dw sediment levels, respectively.

B. Statistical Results

Method: The method used to calculate the 10-day LC_{50} and associated 95% confidence intervals was not reported.

The NOAEC for survival was determined by using a one-way analysis of variance (ANOVA), followed by a Dunnett's test for determination of significance ($p < 0.05$). Prior to analysis, the data were assessed for normality using the Shapiro-Wilk's test and for homogeneity of variance using Levene's test. The survival data were not normally distributed and the variances were not homogeneous; thus, ANOVA was performed on the ranks of the data ($p < 0.05$). The NOAEC was determined by using Fisher's one-tailed exact test. It was determined that the negative control and vehicle control survival values were not significantly different; comparisons were performed using negative control data.

Analyses were performed using SAS statistical software using mean-measured total radioactive residues (TRR) of BAS 500 F (pyraclostrobin) sediment concentrations.

Survival:

LC_{50} : 4.412 mg TRR/kg

95% C.I.: 4.192-4.643 mg TRR/kg

NOAEC: 2.74 mg TRR/kg

LOAEC: 5.05 mg TRR/kg

12. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Survival data were tested to determine if they satisfied the assumptions of normality using Shapiro-Wilks test and homogeneity of variances using Bartlett's test. Survival in the negative and solvent controls was compared using an equal variance two-sample t-test; no difference was detected and all subsequent analyses were conducted using the negative control only. A suggestive decreasing monotonic response was noted, and the Jonckheere-Terpstra Step-Down Test was used to determine the NOAEL/LOAEL values.

The LC50 and 95% confidence interval were determined using the probit analysis. Unless otherwise indicated, effects were considered statistically significant at $p < 0.05$. These analyses were conducted using CETIS version 1.8.7.12 with backend settings approved for use by EFED on 5/29/13. The reviewer relied on the mean-measured sediment (mg TRR/kg), pore and overlying water concentrations to discuss effects in this study.

Survival (sediment):

LC ₅₀ : 4.35 mg TRR/kg	95% C.I.: 3.53-5.23 mg TRR/kg
Slope: 8.72	95% C.I.: 3.46-14
NOAEC: 2.74 mg TRR/kg	
LOAEC: 5.05 mg TRR/kg	

Survival (pore water):

LC ₅₀ : 0.0099 mg TRR/L	95% C.I.: 0.00878-0.011 mg TRR/L
Slope: 9.02	95% C.I.: 5.42-12.6
NOAEC: 0.00625 mg TRR/L	
LOAEC: 0.0115 mg TRR/L	

Survival (overlying water):

LC ₅₀ : 0.00461 mg TRR/L	95% C.I.: 0.00402-0.0052 mg TRR/L
Slope: 8.4	95% C.I.: 4.88-11.9
NOAEC: 0.00282 mg TRR/L	
LOAEC: 0.00541 mg TRR/L	

13. REVIEWER'S COMMENTS:

The reviewer's conclusion LC₅₀ was slightly more conservative (i.e., lower) than the study author's and the reviewer also reported the probit slope. Therefore, the reviewer's results are reported in the Conclusions section of this DER. Results were provided in terms of mean-measured (bulk) sediment concentrations as well as mean-measured pore and overlying water concentrations.

Although it was reported that pore water samples were also analyzed for BAS 500 F (pyraclostrobin), no results were provided.

For HPLC analysis, aqueous samples were acidified to pH \approx 3 with sulfuric acid, and the samples extracted using a C18 SPE cartridge. The SPE cartridge was conditioned twice with 5 mL of ethyl acetate, and then twice with 5 mL of HPLC grade water. The acidified sample extracts were loaded on to the column by passing through the cartridge, which was subsequently rinsed with 5 mL water. The residues were eluted from the column using ethyl acetate (2 x 5 mL). The total ethyl acetate extracts (10 mL) were collected in a culture tube. An aliquot (~2 mL) of the ethyl extract was transferred to a separate culture tube and

evaporated to near dryness. This step was repeated 4 times (i.e. for ~8 mL). The culture tube containing the remaining approximate 2 mL eluent was capped, vortexed and then transferred to the second tube for concentration. Another 1 mL of ACN was added to the collection tube containing the original eluent from the C18 SPE, capped and vortexed once more. The remainder of the extract was transferred to the second tube for concentration, and evaporated to dryness. Acetonitrile (1 mL) was then added to the dry residue, capped, vortexed, sonicated and vortexed to dissolve the residues. After addition of 1 mL of water, the capped culture tube was vortexed thoroughly to obtain homogeneous solution. If necessary, the sample was further diluted with acetonitrile:water (50:50) to provide final sample concentrations within the standard curve range. Samples were analyzed using HPLC with UV (285 nm) detection.

The experimental phase of the definitive study was conducted from November 19 to 29, 2012.

14. REFERENCES:

- U.S. Environmental Protection Agency. 1996. Series 850 - Ecological Effects Test Guidelines, OPPTS 850.1740, Whole Sediment Acute Toxicity Invertebrates, Marine, EPA 712-C-96-355, 12 pp.
- Dinehart, S. In Progress. Rebstock, M. 2011. BAS 500 F: Life-Cycle Toxicity Test of the Saltwater Mysid, *Americamysis bahia*, Conducted Under Flow-Through Conditions. ABC Laboratories, Inc Study Number 68248.

CETIS Summary Report

Report Date: 08 Nov-13 11:23 (p 1 of 1)
Test Code: 49080402 overly | 20-8017-7094

OPPTS 850.1740 Sub-Chronic Sediment (10d SW)

ABC Labs

Batch ID:	01-0355-9818	Test Type:	Sediment Toxicity 10-d	Analyst:	
Start Date:	19 Nov-12	Protocol:	OPPTS 850.1740 Sub-chronic Sediment (1	Diluent:	Artificially Enhanced Seawater
Ending Date:	08 Nov-13 11:11	Species:	Leptocheirus plumulosus	Brine:	Crystal Sea
Duration:	354d 11h	Source:	Chesapeake Cultures	Age:	<4mm
Sample ID:	00-6158-9904	Code:	3ABC990	Client:	CDM Smith
Sample Date:	08 Nov-13 11:11	Material:	Pyraclostrobin	Project:	Fungicide
Receive Date:	08 Nov-13 11:11	Source:	BASF Corporation		
Sample Age:	NA	Station:			

Batch Note: PC Code 099100 MRID 49080402, mean-measured concentrations mg TRR/kg

Point Estimate Summary

Analysis ID	Endpoint	Level	mg/kg se	95% LCL	95% UCL	TU	Method
01-0396-2112	Survival	LC50	0.00461	0.00402	0.0052		Linear Regression (MLE)
21-3434-4305	Survival	LC50	0.00459	0.00436	0.00484		Trimmed Spearman-Kärber

Survival Summary

C-mg/kg sed	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	8	0.975	0.916	1	0.8	1	0.025	0.0707	7.25%	0.0%
0	Negative Control	8	0.994	0.979	1	0.95	1	0.00625	0.0177	1.78%	-1.92%
0.000526		8	0.981	0.95	1	0.9	1	0.0132	0.0372	3.79%	-0.64%
0.00136		8	1	1	1	1	1	0	0	0.0%	-2.56%
0.00282		8	0.969	0.93	1	0.9	1	0.0162	0.0458	4.73%	0.64%
0.00541		8	0.25	0.00729	0.493	0.1	0.95	0.103	0.29	116.0%	74.4%
0.0115		8	0.00625	0	0.021	0	0.05	0.00625	0.0177	283.0%	99.4%

Survival Detail

C-mg/kg sed	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Solvent Blank	1	0.8	1	1	1	1	1	1
0	Negative Control	1	1	1	1	0.95	1	1	1
0.000526		1	1	1	1	0.95	0.9	1	1
0.00136		1	1	1	1	1	1	1	1
0.00282		1	1	1	1	0.9	1	0.9	0.95
0.00541		0.1	0.1	0.15	0.15	0.95	0.3	0.15	0.1
0.0115		0	0.05	0	0	0	0	0	0

CETIS Summary Report

Report Date: 29 Oct-13 12:09 (p 1 of 1)
Test Code: 49080402 porewt | 09-2813-9950

OPPTS 850.1740 Sub-Chronic Sediment (10d SW)

ABC Labs

Batch ID: 04-6072-9612 Test Type: Sediment Toxicity 10-d Analyst:
Start Date: 09 Nov-12 Protocol: OPPTS 850.1740 Sub-chronic Sediment (1 Diluent: Artificially Enhanced Seawater
Ending Date: 28 Oct-13 20:40 Species: Leptocheirus plumulosus Brine: Crystal Sea
Duration: 353d 21h Source: Chesapeake Cultures Age:

Sample ID: 07-5981-9255 Code: 49080402 porewt Client: CDM Smith
Sample Date: 19 Nov-12 Material: Pyraclostrobin Project: Fungicide
Receive Date: 28 Oct-13 20:40 Source: BASF Corporation
Sample Age: NA Station:

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
15-0313-3094	Survival	0	>0		5.56%		Equal Variance t Two-Sample Test
10-1479-3675	Survival	0.00625	0.0115	0.008478	10.8%		Mann-Whitney U Two-Sample Test

Point Estimate Summary

Analysis ID	Endpoint	Level	mg/kg se	95% LCL	95% UCL	TU	Method
11-6101-4854	Survival	LC5	0.0065	0.00467	0.00761		Linear Regression (MLE)
		LC10	0.00713	0.00542	0.00818		
		LC15	0.00759	0.00599	0.0086		
		LC20	0.00798	0.00646	0.00896		
		LC25	0.00833	0.0069	0.0093		
		LC40	0.00928	0.00806	0.0103		
		LC50	0.0099	0.00878	0.011		
00-4467-5484	Survival	LC50	0.00985	0.00939	0.0103		Trimmed Spearman-Kärber

Survival Summary

C-mg/kg sed	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	8	0.975	0.916	1	0.8	1	0.025	0.0707	7.25%	0.0%
0	Negative Control	8	0.994	0.979	1	0.95	1	0.00625	0.0177	1.78%	-1.92%
0.00169		8	0.981	0.95	1	0.9	1	0.0132	0.0372	3.79%	-0.64%
0.00338		8	1	1	1	1	1	0	0	0.0%	-2.56%
0.00625		8	0.969	0.93	1	0.9	1	0.0162	0.0458	4.73%	0.64%
0.0115		8	0.25	0.00729	0.493	0.1	0.95	0.103	0.29	116.0%	74.4%
0.0229		8	0.00625	0	0.021	0	0.05	0.00625	0.0177	283.0%	99.4%

Survival Detail

C-mg/kg sed	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Solvent Blank	1	0.8	1	1	1	1	1	1
0	Negative Control	1	1	1	1	0.95	1	1	1
0.00169		1	1	1	1	0.95	0.9	1	1
0.00338		1	1	1	1	1	1	1	1
0.00625		1	1	1	1	0.9	1	0.9	0.95
0.0115		0.1	0.1	0.15	0.15	0.95	0.3	0.15	0.1
0.0229		0	0.05	0	0	0	0	0	0

CETIS Summary Report

Report Date: 29 Oct-13 11:58 (p 1 of 1)

Test Code: 099100 49080402 | 19-5701-3609

OPPTS 850.1740 Sub-Chronic Sediment (10d SW)

ABC Labs

Batch ID: 01-0355-9818	Test Type: Sediment Toxicity 10-d	Analyst:
Start Date: 19 Nov-12	Protocol: OPPTS 850.1740 Sub-chronic Sediment (1	Diluent: Artificially Enhanced Seawater
Ending Date:	Species: Leptocheirus plumulosus	Brine: Crystal Sea
Duration: NA	Source: Chesapeake Cultures	Age: <4mm
Sample ID: 08-9245-1038	Code: 49080402 sedmnt	Client: CDM Smith
Sample Date: 19 Nov-12	Material: Pyrethroids	Project: Fungicide
Receive Date:	Source: BASF Corporation	
Sample Age: NA	Station:	

Batch Note: PC Code 099100 MRID 49080402, mean-measured concentrations mg TRR/kg

Sample Note: PC Code 099100 MRID 49080402

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
00-1127-0252	Survival	0	>0		5.56%		Equal Variance t Two-Sample Test
18-3036-7824	Survival	2.74	5.05	3.72	NA		Jonckheere-Terpstra Step-Down Test
19-7498-1714	Survival	2.74	5.05	3.72	10.8%		Mann-Whitney U Two-Sample Test

Point Estimate Summary

Analysis ID	Endpoint	Level	mg/kg se	95% LCL	95% UCL	TU	Method
00-9431-0527	Survival	LC5	2.82	1.35	3.49		Linear Regression (MLE)
		LC10	3.1	1.7	3.74		
		LC15	3.31	1.98	3.93		
		LC20	3.48	2.24	4.1		
		LC25	3.64	2.47	4.26		
		LC40	4.07	3.12	4.78		
		LC50	4.35	3.53	5.23		
14-9719-7749	Survival	LC50	4.39	4.17	4.62		Trimmed Spearman-Kärber

Survival Summary

C-mg/kg sed	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	8	0.975	0.916	1	0.8	1	0.025	0.0707	7.25%	0.0%
0	Negative Control	8	0.994	0.979	1	0.95	1	0.00625	0.0177	1.78%	-1.92%
0.771		8	0.981	0.95	1	0.9	1	0.0132	0.0372	3.79%	-0.64%
1.51		8	1	1	1	1	1	0	0	0.0%	-2.56%
2.74		8	0.969	0.93	1	0.9	1	0.0162	0.0458	4.73%	0.64%
5.05		8	0.25	0.00729	0.493	0.1	0.95	0.103	0.29	116.0%	74.4%
11.3		8	0.00625	0	0.021	0	0.05	0.00625	0.0177	283.0%	99.4%

Survival Detail

C-mg/kg sed	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Solvent Blank	1	0.8	1	1	1	1	1	1
0	Negative Control	1	1	1	1	0.95	1	1	1
0.771		1	1	1	1	0.95	0.9	1	1
1.51		1	1	1	1	1	1	1	1
2.74		1	1	1	1	0.9	1	0.9	0.95
5.05		0.1	0.1	0.15	0.15	0.95	0.3	0.15	0.1
11.3		0	0.05	0	0	0	0	0	0